Applied Biosystems

VLA TaqMan® Influenza A/H5/H7 Detection Kits

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For The VLA TaqMan® Influenza A/H5/H7 Detection Kits

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VLA TaqMan® Influenza A/H5/H7 Detection Kits

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Contents

Preface vii
Safety information vii
How to use this guide viii
How to obtain support viii

Protocol VLA TaqMan® Influenza A/H5/H7 Detection Kits 1
Product overview 1
Chemistry overview 2
Materials and equipment 5
Detection procedure workflow 9
Isolate RNA 10
Prepare the plate document 10
Prepare the RT-PCR reactions 12
Run the reactions 16
View and verify results 18
Troubleshooting 23

Appendix A Good PCR Practices 25
Prevent contamination and nonspecific amplification 25

Appendix B Safety 27
Chemical safety 28
Chemical waste safety 29
Biological hazard safety 31
Chemical alerts 31

Documentation 33
Related documentation 33

Safety information

Note: For general safety information, see this Preface and Appendix B, "Safety" on page 27. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Refer to the Instrument User Manual provided with the relevant instrument, for user-related instrument safety information.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see Appendix B.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

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At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

VLA TaqMan® Influenza A/H5/H7 Detection Kits

Product overview

Product description

The VLA TaqMan® Influenza A/H5/H7 Detection Kits, in a lyophilized reagent format, rapidly assay samples for the presence of Influenza type A. The kits were validated by the UK Veterinary Laboratory Agency (VLA) to detect the same strains as the existing VLA assays.

The VLA TaqMan® Influenza A/H5/H7 Detection Kits reactions use:

- Reverse transcription to convert viral RNA to cDNA.
- Polymerase chain reaction (PCR) to amplify the viral target.
- TaqMan® probes to detect the presence of a specific influenza gene sequence.
- An Internal Positive Control (IPC) to check for the presence of PCR-inhibitors.
- One of the following Applied Biosystems instruments to perform the PCR and detect the probe cleavage:
  - Applied Biosystems 7500 Fast Real-Time PCR System with SDS software version 1.3 or 1.4
  - Applied Biosystems 7300 or 7500 Real-Time PCR System
  - ABI PRISM® 7000 Sequence Detection System
  - Applied Biosystems 7900HT Fast Real-Time PCR System (using a standard block)
  - Applied Biosystems StepOne™ or StepOnePlus™ Real-Time PCR System

The TaqMan® primers and probes in the VLA TaqMan® Influenza A/H5/H7 Detection Kits are based on the existing VLA assay sequences for Influenza A, Influenza A subtype H5, and Influenza A subtype H7. Specifically, the kits were validated by the VLA to detect the following:

- Detection Kit A: all avian influenza A virus subtypes (H1 through H16) from all geographic regions
- Detection Kit H5: Eurasian H5 avian influenza virus subtypes
- Detection Kit H7: Eurasian H7 avian influenza virus subtypes

Ongoing validation monitors the performance of the test against new isolates.

Chemistry overview

Reaction components

Reaction components of each of the VLA TaqMan® Influenza A/H5/H7 Detection Kits include:

- Assay Beads (Influenza A, Influenza A subtype H5, or Influenza A subtype H7) containing enzymes, primers, probes, and other components for reverse transcription (RT) and polymerase chain reaction (PCR).
- Sample:
  - RNA, which you supply, isolated from environmental or epidemiological samples
  - RNA positive control
  - Negative controls

Each kit provides reagents for 96 reactions.

About negative and positive controls

It is good practice to include PCR-specific and process-specific controls in your experiment. A negative PCR control is a reaction solution that lacks a target sequence. At least one negative control is required for every run, to monitor for reagent integrity and contamination (unexpected amplification in the absence of a target). A negative control in the form of nuclease-free water (PN 4340451) is provided in the kit.

A positive PCR control contains a template known to be amplified by the primers in the assay. In RT-PCR the control template is an RNA. Target signal (FAM™ dye) that is not detected in a positive-control well indicates a pipetting error or a problem with amplification. Positive RNA controls for Influenza A (PN 4400534), the H5 subtype of Influenza A (PN 4400535), or the H7 subtype of Influenza A (PN 4400536) are provided in the kit. At least one positive control is required per run.

- Negative controls:
  - Run a PCR control (water only, no template) to confirm that kit reagents do not contain any components that amplify.
  - Run a process control to confirm that your process does not contain any components that amplify from a sample with no virus.
- Positive controls:
  - Run a PCR control to confirm that the kit reagents and instrument function to amplify the expected target. A synthetic RNA is provided as a positive control in the kit.
  - Run a process control to test the entire process from sample preparation to detection to ensure that your process can generate a positive result from a sample known to contain virus.

IMPORTANT! When you run positive controls, cover the sample and negative control wells before pipetting positive controls, to avoid cross-contamination of samples and negative controls.

Internal positive control (IPC)

Applied Biosystems includes an IPC (VIC® dye) in the assay beads. A positive IPC signal:

- Demonstrates that PCR reagents amplify as expected.
- Allows accurate interpretation of negative sample results.

RT-PCR

The VLA TaqMan® Influenza A and Influenza A subtype H5 and H7 reactions use a one-step reverse transcription-polymerase chain reaction (RT-PCR) format in a single-tube. The assay beads contain a thermoreactive reverse transcriptase and a thermostable DNA polymerase that provide the 5′ to 3′ nuclease activity necessary for cleavage of the fluorogenic probe.

During RT-PCR (see Figure 1 on page 4):

RNA is reverse-transcribed to cDNA.

PCR cycles are performed.

1. The primers anneal and extend, and the probe hybrizes.
   a. The DNA polymerase creates a new DNA strand by extending the primers with nucleotides.
   b. If the cDNA target of interest is present in the amplification product, the TaqMan probe hybridizes to the sequence.
2. The DNA polymerase cleaves the probe.
   a. The 5′ to 3′ nucleolytic activity of the DNA polymerase cleaves the hybridized probe between the reporter dye and the quencher dye (see TaqMan probes below for more information). The reporter dye fragments are displaced from the target, resulting in an increase in fluorescence.

      Note: This step, which occurs in every cycle, does not interfere with the exponential accumulation of product.

   b. The polymerization of the strand continues. The 3′ end of the probe is blocked to prevent extension of the probe during PCR.
3. Fluorescence increases. The accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye.

   Note: The increase in fluorescence signal occurs only if the target sequence is complementary to the probe and is amplified during PCR. Nonspecific amplification is not detected in the absence of a probe-binding site.

TaqMan® probes

The TaqMan® probe contains a fluorescent reporter dye at the 5′ end of the probe and a quencher dye at the 3′ end of the probe. When the probe is intact, the proximity of the reporter dye to the quencher dye suppresses the reporter fluorescence. Probe cleavage during the PCR reaction spatially separates the reporter dye from the quencher moiety and allows detection of the reporter dye fluorescence.

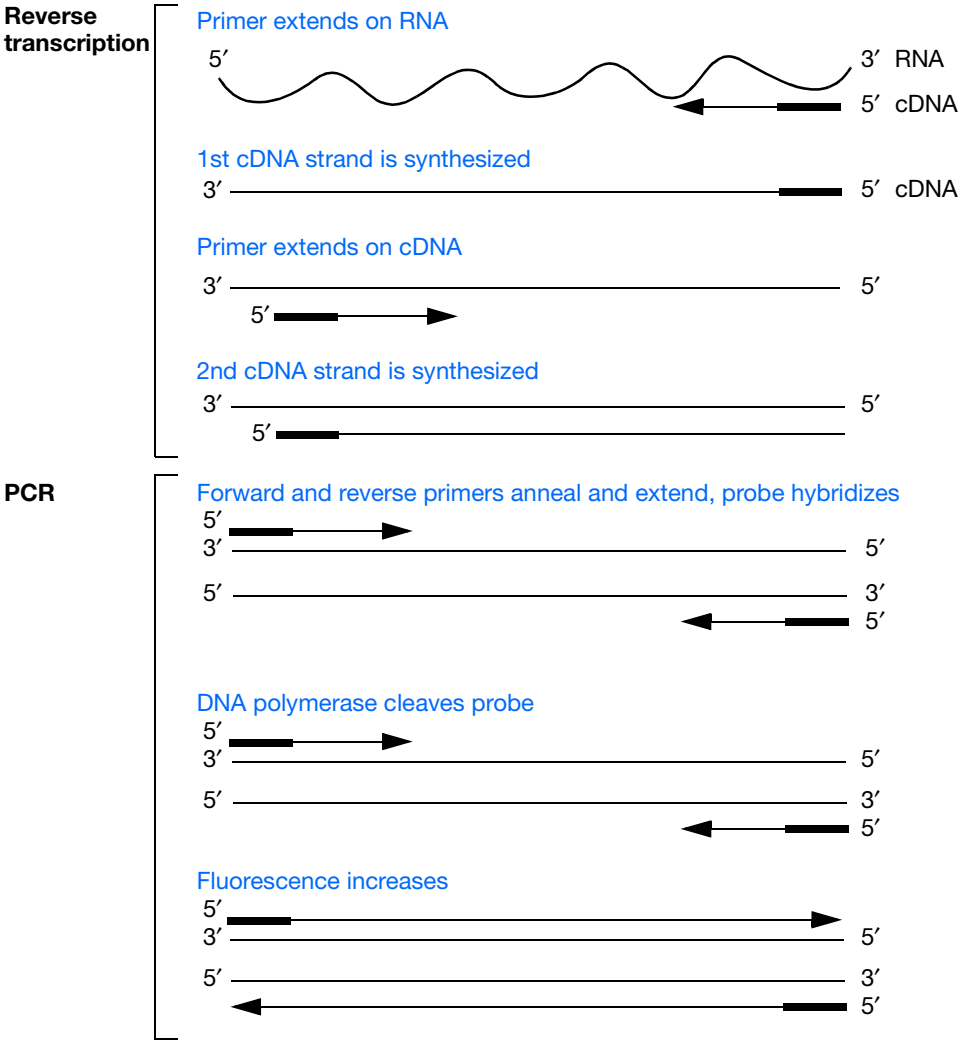


Figure 1 Reverse transcription–polymerase chain reaction (RT-PCR)

Materials and equipment

Kit contents

The VLA TaqMan® Influenza A/H5/H7 Detection Kits contain reagents for 96 Influenza A, or Influenza A subtype H5 or subtype H7 RT-PCR reactions. Each kit contains:

- This protocol
- One pouch containing:
  - Assay beads (in tubes) for Influenza A or Influenza A subtype H5, or H7
  - One bag of 12 MicroAmp® Optical 8-Cap Strips
- One box containing:
  - RNA Positive Control: Influenza A, Influenza A subtype H5, or Influenza A subtype H7
  - Negative control: nuclease-free water

Table 1 VLA TaqMan Influenza A/H5/H7 Detection Kits

Item	Cap Color	Reagent
VLA TaqMan® Influenza A Detection Kit (PN 4398276)		
VLA TaqMan® Influenza A Detection Kit (Part 1 of 2) (PN 4398293)	Dark Green	VLA Influenza A Assay Beads, 96 tubes in 8-tube strips (96 reactions/kit), twelve 8-tube strips
	N/A	MicroAmp® Optical 8-Cap Strips, 96 caps in strips of 8
VLA TaqMan® Influenza A Detection Kit Controls (Part 2 of 2) (PN 4398280)	Grey	Negative Control, nuclease-free water, 2 tubes, 2.0 mL each (PN 4340451)
	Red	VLA RNA Positive Control, Influenza A, 1000 copies/µL, 1 tube, 1.5 mL (PN 4400534)
VLA TaqMan® Influenza A Subtype H5 Detection Kit (PN 4398312)		
VLA TaqMan® Influenza A Subtype H5 Detection Kit (Part 1 of 2) (PN 4398278)	Orange	VLA Influenza A Subtype H5 Assay Beads, 96 tubes in 8-tube strips (96 reactions/kit)
	N/A	MicroAmp® Optical 8-Cap Strips, 96 caps in strips of 8
VLA TaqMan® Influenza A Subtype H5 Detection Kit Controls (Part 2 of 2) (PN 4398319)	Grey	Negative Control, nuclease-free water, 2 tubes, 2.0 mL each (PN 4340451)
	Yellow	VLA RNA Positive Control H5, Influenza A subtype H5, 1000 copies/µL, 1 tube, 1.5 mL (PN 4400535)
VLA TaqMan® Influenza A Subtype H7 Detection Kit (PN 4398277)		

Table 1 VLA TaqMan Influenza A/H5/H7 Detection Kits

Item	Cap Color	Reagent
VLA TaqMan® Influenza A Subtype H7 Detection Kit (Part 1 of 2) (PN 4398295)	Blue	VLA Influenza A Subtype H7 Assay Beads, 96 tubes in 8-tube strips (96 reactions/kit)
	N/A	MicroAmp® Optical 8-Cap Strips, 96 caps in strips of 8
VLA TaqMan® Influenza A Subtype H7 Detection Kit Controls (Part 2 of 2) (PN 4398321)	Grey	Negative Control, nuclease-free water, 2 tubes, 2.0 mL each (PN 4340451)
	Green	VLA RNA Positive Control H7, Influenza A subtype H7, 1000 copies/µL, 1 tube, 1.5 mL (PN 4400536)

Storage

- Upon receipt, store Assay Beads at 2 °C to 8 °C. Protect from light and moisture.
- Upon receipt, store all other components at –15 °C to –25 °C.
- Minimize freeze-thaw cycles.
- Protect assay components from light. Excessive exposure to light may affect the fluorescent probes.

Materials not included in the kit

The following table includes materials required for using (but not included in) the VLA TaqMan® Influenza A/H5/H7 Detection Kits. Unless otherwise noted, many of the listed items are available from major laboratory suppliers (MLS).

Table 2 Instruments, equipment, consumables, and reagents

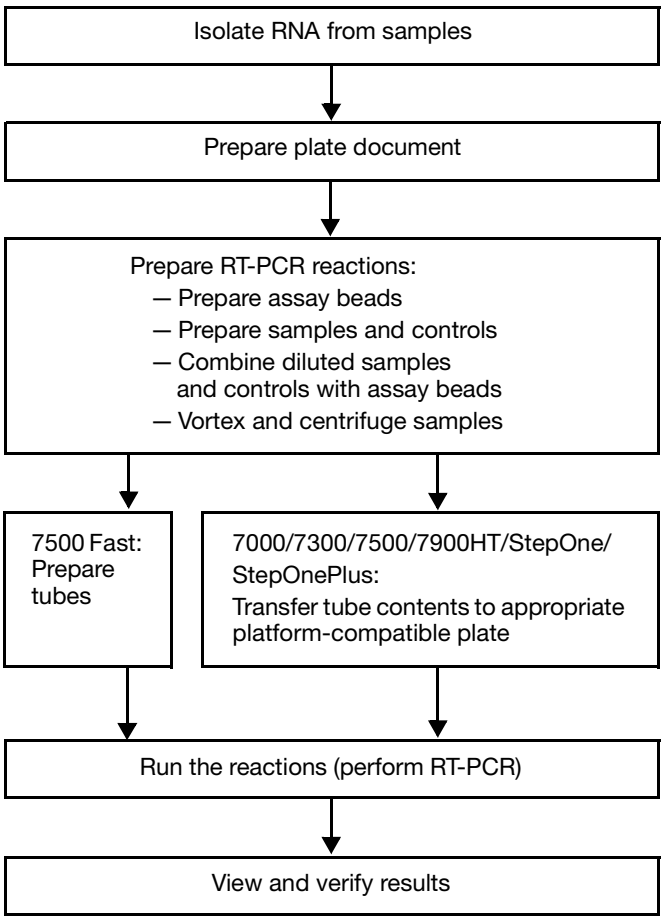
Item	Source
Applied Biosystems Instruments	
ABI PRISM® 7000 Sequence Detection System	Contact your local Applied Blosystems sales office.
Applied Biosystems 7300 Real-Time PCR System	
Applied Biosystems 7500 Real-Time PCR System	
Applied Biosystems 7500 Fast Real-Time PCR System	
Applied Biosystems 7900HT Real-Time PCR System (using a standard block)	
StepOne™ Real-Time PCR System	
StepOnePlus™ Real-Time PCR System	
Equipment	
96-well cold block	MLS
Benchtop microcentrifuge	MLS
Centrifuge with adapter for 96-well plate	MLS
Ice bucket	MLS
MicroAmp® Adhesive Film Applicator	Applied Biosystems PN 4333183
MicroAmp® Optical 48-Well Base Adaptor	Applied Biosystems PN 4375284 For use with StepOne™ system reactions.
MicroAmp® Splash Free 96-Well Base	Applied Biosystems PN 4312063
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
7500 Fast System Precision Plate Holder for MicroAmp® Tube Strips	Applied Biosystems PN 4403809‡
Vortexer	MLS
Consumables	
Disposable gloves	MLS

Table 2 Instruments, equipment, consumables, and reagents *(continued)*

Item	Source
Plates, tubes, caps, and covers as needed	MLS
RNase-free microfuge tubes, 1.5 mL	Applied Biosystems PN AM12400
RNase-free tips, 200 µL	Applied Biosystems PN AM12650
RNase-free tips, 1000 µL	Applied Biosystems PN AM12660
MicroAmp® Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.2 mL	Applied Biosystems PN 4306737 Not recommended for use with 7500 Fast System. For 7500 Fast System reactions, use PN 4346906.
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.1 mL	Applied Biosystems PN 4346906
MicroAmp® Optical 8-Tube Strip, 1000 tubes in strips of 8, 0.2 mL	Applied Biosystems PN 4316567 Not recommended for use with 7500 Fast System. For 7500 Fast System reactions, use PN 4358293.
MicroAmp® Fast 8-Tube Strip, 0.1 mL	Applied Biosystems PN 4358293
MicroAmp® Optical 8-Cap Strip, 300 strips	Applied Biosystems PN 4323032
MicroAmp® Fast Optical 48-Well Reaction Plate, 20 plates, 0.1 mL	Applied Biosystems PN 4375816 For use with StepOne™ System reactions.
MicroAmp® Optical 48-Well Adhesive Film	Applied Biosystems PN 4375323 For use with StepOne system reactions.
MicroAmp® Optical 96-Well Reaction Plate with Barcode and Optical Adhesive Films, 100 plates and 100 covers	Applied Biosystems PN 4314320
MicroAmp® Optical Adhesive Film, 25 covers	Applied Biosystems PN 4360954
MicroAmp® Optical Adhesive Film Kit	Applied Biosystems PN 4313663
Reagents	
Ambion RT-PCR Grade Water	Applied Biosystems PN AM9935

‡ Includes recommended usage procedure

Detection procedure workflow


Isolate RNA from samples
Prepare plate document
Prepare RT-PCR reactions:
— Prepare assay beads
— Prepare samples and controls
— Combine diluted samples and controls with assay beads
— Vortex and centrifuge samples
7500 Fast: Prepare tubes
7000/7300/7500/7900HT/StepOne/ StepOnePlus:
Transfer tube contents to appropriate platform-compatible plate
Run the reactions (perform RT-PCR)
View and verify results

Isolate RNA

Validating your own RNA sample preparation procedure

Use high purity viral RNA that is free of materials that can inhibit amplification in the performance of these assays. Most commercially available viral RNA isolation kits satisfy the requirements of the VLA TaqMan® Influenza A/H5/H7 Detection Kits. However, Applied Biosystems has not validated an RNA isolation procedure for use with this protocol. You must validate your own procedure.

Sample handling precautions

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR 1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

WARNING! CHEMICAL HAZARD. VLA TaqMan® Influenza A/H5/H7 Detection Kits.

Prepare the plate document

1. Create a plate document (select **Absolute Quantification (Standard Curve)** from Assay drop-down list). For information on creating a plate document, refer to the documentation provided with your instrument.

2. Create or select FAM and VIC dye detectors with the Quencher Dye set to (**none**) or (**Non Fluorescent**).

3. Associate both FAM and VIC dye detectors with each reaction.

   Note: The FAM dye is used for detection of the target (for example, Influenza A subtype H7) and the VIC dye is used for detection of the internal positive control (IPC).

4. Set thermal cycling conditions according to the following table:

Step	RT		PCR	
			CYCLE (each of 40 cycles)	
	HOLD	HOLD	Denature	Anneal/extend
Temp	45 °C	95 °C	95 °C	54 °C
Time	30 min	2 min	15 sec	1 min

5. Set Sample Volume to **30** µL.

6. Select the appropriate Run Mode for your system according to the following table:

System	Run mode
7500 and 7500 Fast	Standard 7500
7000	Standard
7300	Standard
7900HT	Standard
StepOne	Standard
StepOnePlus	Standard

Prepare the RT-PCR reactions

Prepare the assay beads

1. Open the storage pouch containing the assay beads by cutting at the notch located in the upper corner of the storage pouch above the zip lock strip.

   IMPORTANT! Do not remove the desiccant from the storage pouch.

2. Remove the appropriate number of individual tubes or 8-tube strips: one tube for each reaction you plan to run.

   IMPORTANT! Applied Biosystems recommends performing three replicates for each sample.

3. Place tubes on ice or in cold block.

4. Seal the storage pouch using the zip lock strip and store at 2 °C to 8 °C.

Prepare samples and controls

Nuclease-free water is provided as a negative control and to dilute samples and controls. The recommended RNA concentration is between 100 to 10,000 copies per reaction.

1. Thaw all reaction components completely.

   IMPORTANT! Keep all tubes on ice.

2. For each type of sample or control to be analyzed, label a sterile microcentrifuge tube with the sample name.

3. Calculate the volume of components needed for the number of diluted samples, sample replicates, and controls for one assay. The table below lists volumes for three sample types: unknown RNA sample, negative control, and RNA positive control. Multiply volumes by an appropriate factor for multiple replicates.

   Note: Applied Biosystems recommends performing three replicates for each sample, the positive control, and the no template control (30 µL total volume per tube).

Component	Unknown RNA		RNA positive control		Negative control	
	1 replicate	4 replicates	1 replicate	4 replicates	1 replicate	4 replicates
Sample	10 µL	40 µL	10 µL	40 µL	–	–
Nuclease-free water	20 µL	80 µL	20 µL	80 µL	30 µL	120 µL
Total	30 µL	120 µL	30 µL	120 µL	30 µL	120 µL

4. Prepare the reagent mix:
   a. Pipette the appropriate volumes of components into the labeled sample tube from step 2 on page 12.
   b. Mix the components by vortexing.
   c. Briefly spin down tube contents using a microcentrifuge.

IMPORTANT! Keep all tubes on ice.

5. Repeat step 3 and step 4 above to prepare a reagent mix for each remaining sample or control.

Combine diluted samples and controls with assay beads

1. Examine the assay beads in the 8-tube strips. Gently tap the tubes as needed to move all assay beads to the bottom of all tubes.
2. Carefully remove the colored caps from the 8-tube strips containing the assay beads to avoid disturbing the beads from the bottom of the tubes. Discard the colored caps.

IMPORTANT! Avoid disturbing the beads from the bottom of the tubes.

IMPORTANT! Do not use colored caps or tubes for kit reactions. Colored caps or tubes are not compatible with real-time PCR.

3. For each sample or control, transfer 30 µL of the reagent mix prepared above in step 4, into a tube containing the appropriate influenza assay bead. Beads dissolve in 1 to 5 seconds.

IMPORTANT! Use a new pipette tip for each sample.

Next step

After you have combined the assay beads with the samples:

- Prepare tubes for the 7500 Fast System (7500 Fast System only) as described on page 14.

Or:

- Select the appropriate optical plate for your system and proceed to "Transfer tube contents to a plate and seal the plates" on page 15:
  - For 7000, 7300, 7500, 7900HT, StepOnePlus™ Systems, transfer the reaction contents from the assay bead tubes to a 96-well standard optical plate (PN 4306737).
  - For the StepOne™ System, transfer the reaction contents from the assay bead tubes to a 48-well optical plate (PN 4375816).

Prepare tubes for the 7500 Fast System (7500 Fast System only)

To prepare tubes for the 7500 Fast System:

IMPORTANT! 8-tube strips containing assay beads are compatible only with 7500 Fast instruments.

1. Cap the tubes. Seal each tube completely with the transparent optical strip caps provided in the kit (PN 4316567).

   Follow the following instructions carefully to reduce the risk of tubes collapsing when using the MicroAmp® Fast 8-Tube strips (0.1 mL).

   To minimize bending or misaligning the tube strips during the capping process, place the tube strips in the MicroAmp® 96-Well Base (PN N8010531) and use the MicroAmp® Cap Installing Tool (Handle, PN 4330015) to cap the tubes. Verify that the strips are straight and that each tube is in line with the adjacent tube.

2. Vortex the tubes on the high setting for 5 seconds.

   IMPORTANT! Ensure that the caps are on firmly before vortexing.

3. Spin down tube contents at 2000 × g for 20 seconds using a centrifuge with a plate adapter.

   IMPORTANT! Make sure samples are thoroughly mixed.

   IMPORTANT! Make sure reagents are in the bottom of the wells after centrifugation.

4. Proceed to "Run the reactions" on page 16.

Transfer tube contents to a plate and seal the plates

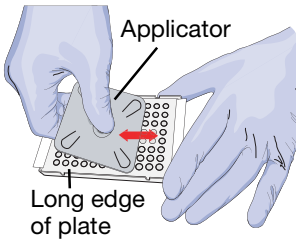
1. Verify that the reaction mix is at the bottom of each tube. If not, cap the tubes securely, vortex them, and spin them as described in "Prepare tubes for the 7500 Fast System (7500 Fast System only)" on page 14. Remove and discard the strip caps.

2. Use a pipette to transfer the entire reaction volume of each sample or control to the appropriate plate.

   IMPORTANT! Use a new pipette tip for each reaction, even when pipetting the same sample or control.

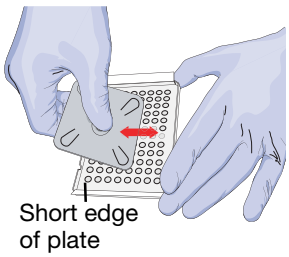
   IMPORTANT! Keep all tubes and plates on ice.

3. Place an optical adhesive cover (PN 4314320) on the plate, then rub the flat edge of the applicator (PN 4333183) back and forth along the *long* edge of the plate.

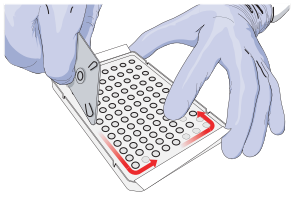
   IMPORTANT! Apply significant downward pressure on the applicator to completely seal the wells. Pressure is required to activate the adhesive on the optical cover.

4. Rub the flat edge of the applicator back and forth along the *short* edge (width) of the plate.

5. Rub the edge of the applicator horizontally and vertically between all wells.

6. Rub the edge of the applicator around all outside edges of the plate using small back and forth motions to completely seal around the outside wells.

7. Vortex the plate on the low setting for 5 seconds.

8. Spin down the plate at 2000 × g for 20 seconds using a centrifuge with a plate adapter.

   IMPORTANT! Make sure reagents are in the bottom of the wells.

9. Proceed to "Run the reactions" on page 16.

Run the reactions

Overview

Running tubes or a plate consists of using an Applied Biosystems Sequence Detection System (SDS) or Real-Time PCR System to analyze your sample.

Before you begin

Ensure that your instrument is properly installed and calibrated. For calibration information, see the documentation provided with your instrument.

Run the 8-tube reactions (7500 Fast Systems only)

For 8-tube strips in a 7500 Fast System:

Use of the 7500 Fast Precision Plate Holder for MicroAmp® Tube Strips (PN 4403809) is optional.

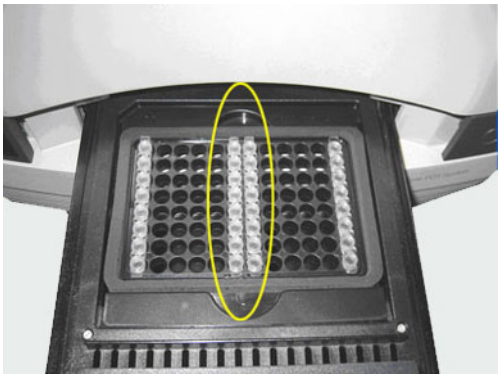
When using the MicroAmp 8-tube strips, if columns 1 and 12 are not used, insert two fully capped empty 8-tube strips into the far left and far right columns.

Note: The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby minimizing the risk of tubes collapsing.

1. Carefully insert, in pairs, the 8-tube strips containing samples starting from the center of the plate holder and moving out.

   Note: A minimum of two and a maximum of 12 tube strips can be run at one time.

   IMPORTANT! Always use intact 8-tube strips. If fewer samples are needed, transfer contents to an intact 8-tube strip. It is alright to have empty wells.



2. Open the plate document that corresponds to the reaction plate created ("Prepare the plate document" on page 10).

3. Start the run.

Run the 48- or 96-well plate reactions

1. Place the appropriate plate adaptor in the Real-Time PCR System.

2. Load the reaction plate into the Real-Time PCR System.

3. Open the plate document that corresponds to the reaction plate created in ("Prepare the plate document" on page 10).

4. Start the run.

View and verify results

Overview

The steps you perform to view results depend on the instrument you use. Refer to the appropriate instrument user guide for instructions on how to analyze data and view your results.

View results

1. View the amplification plots for all reactions.
2. For all reactions, under Analysis Settings:
   - Select **Manual Baseline**.
   - Set Start (cycle) to **6**.
   - Set End (cycle) to **15**.
   - Set **Manual C_T** threshold to **0.20**.
   - Click **Analyze**.

Verify internal positive control (IPC) results

Examine the IPC signal (VIC® dye detector) in all wells. For most instruments, the IPC signal for the internal positive control in all wells should yield C_Ts in the range of 28 to 36 at the manual C_T threshold of 0.2.

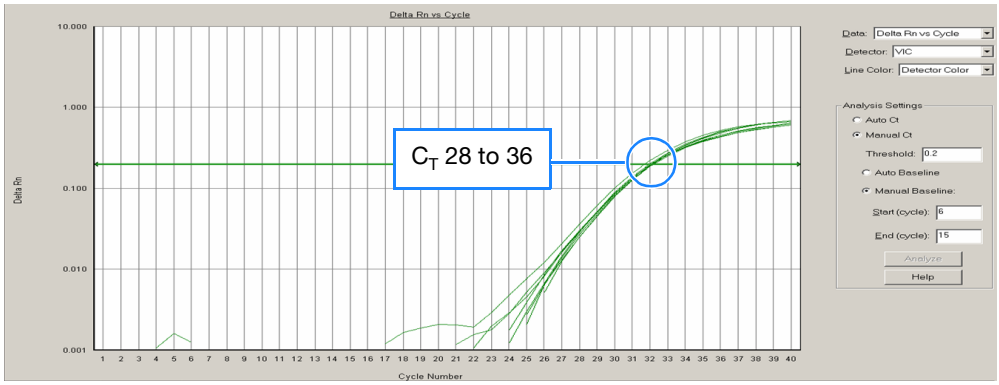


Figure 2 Amplification plot of IPC (VIC® dye detector) in negative controls for the Influenza A assay on the 7500 Fast Real-Time PCR System; 8 replicates. The circled area indicates where the signal crosses the C_T threshold (horizontal green line).

If you observe any of the following, see "Troubleshooting" on page 23:

- IPC signal is not present in negative control, unknown, or positive control wells
- IPC signal C_T is greater than 36 in any well
- IPC signal C_T is inconsistent between replicate wells

Interpret results of unknown samples

Examine the target-specific signal (FAM™ dye detector) in all wells. Use the following tables to interpret the results. Table information applies to results generated on any Applied Biosystems Real-Time PCR System.

Table 3 Analysis for Influenza A assay

FAM signal (target- specific)	FAM C_T value	VIC signal (IPC)	Result	Example
Present, with sigmoidal character	≤36	Present	Positive	Figure 3
Present, with linear character	<40	Present	Negative	Figure 4
Present, with sigmoidal character	>36	Present	Indeterminate – repeat assay	Figure 5
Present, with sigmoidal character	≤36	Absent	Invalid – repeat assay	Figure 6
Absent	–	Present	Negative	Figure 7
Absent	–	Absent	Invalid – repeat assay	–

Table 4 Analysis for Influenza subtype H5 and H7 assays

FAM signal (target- specific)	FAM C_T value	VIC signal (IPC)	Result	Example
Present, with sigmoidal character	≤35	Present	Positive	Figure 3
Present, with linear character	<40	Present	Negative	Figure 4
Present, with sigmoidal character	>35	Present	Indeterminate – repeat assay	Figure 5
Present	≤35	Absent	Invalid – repeat assay	Figure 6
Absent	–	Present	Negative	Figure 7
Absent	–	Absent	Invalid – repeat assay	–

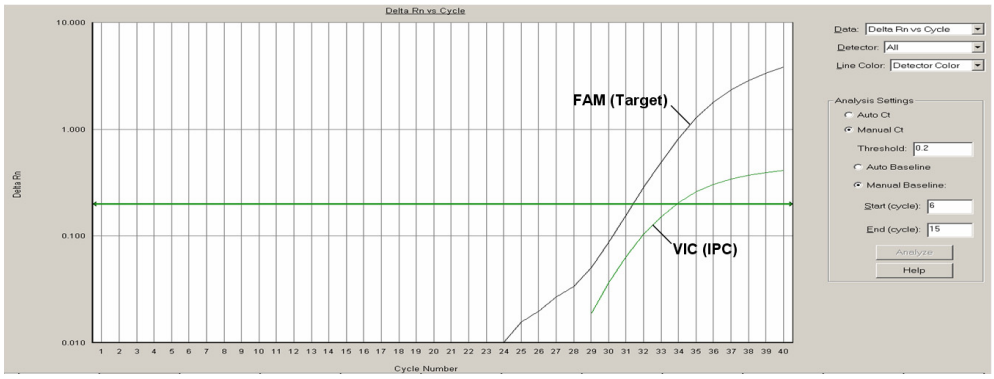


Figure 3 Positive result: FAM and VIC are present with sigmoidal character and the FAM signal crosses the threshold before the cutoff value (C_T ≤36 for Influenza A and C_T ≤35 for Influenza A subtypes H5 and H7). Amplification plots of target-specific FAM and IPC-specific VIC signals were generated on the 7500 Fast System.

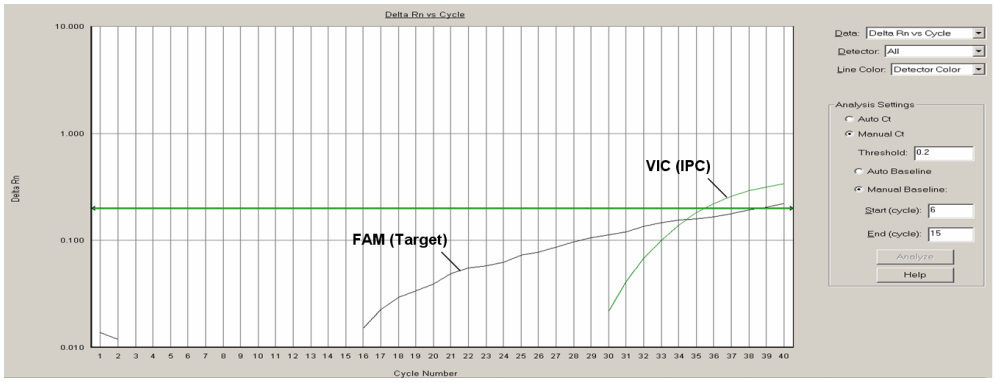


Figure 4 Negative result: FAM present with linear character. Amplification plots of target-specific FAM and IPC-specific VIC signals were generated on the 7500 Fast System.

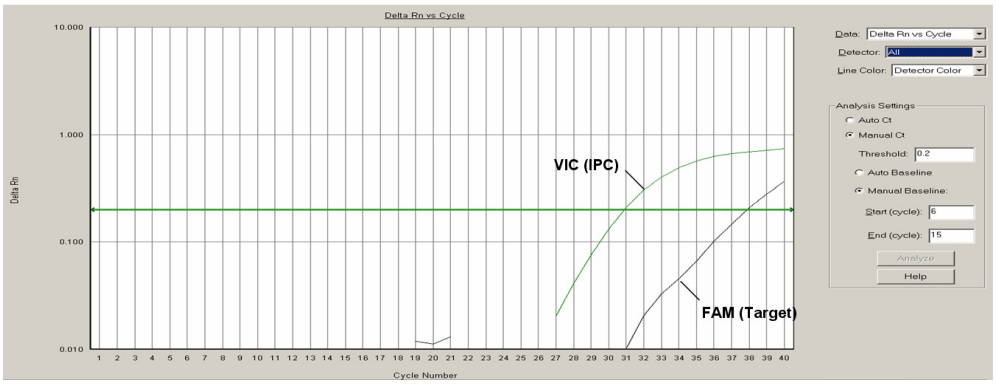


Figure 5 Indeterminate result: FAM C_T >36 for Influenza A, FAM C_T >35 for either VLA H5 or H7 assays (repeat assay). Amplification plots of target-specific FAM and IPC-specific VIC signals were generated on the 7500 Fast System.

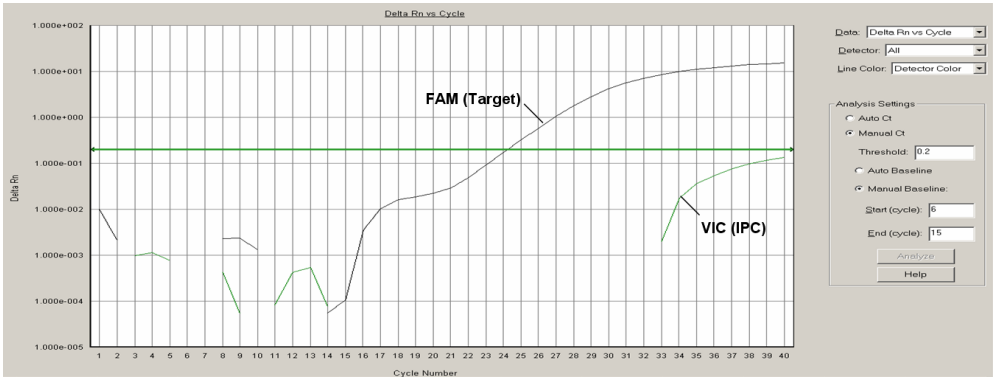


Figure 6 Invalid result: FAM present, VIC absent (repeat assay). Amplification plots of target-specific FAM and IPC-specific VIC signals were generated on the 7500 Fast System.

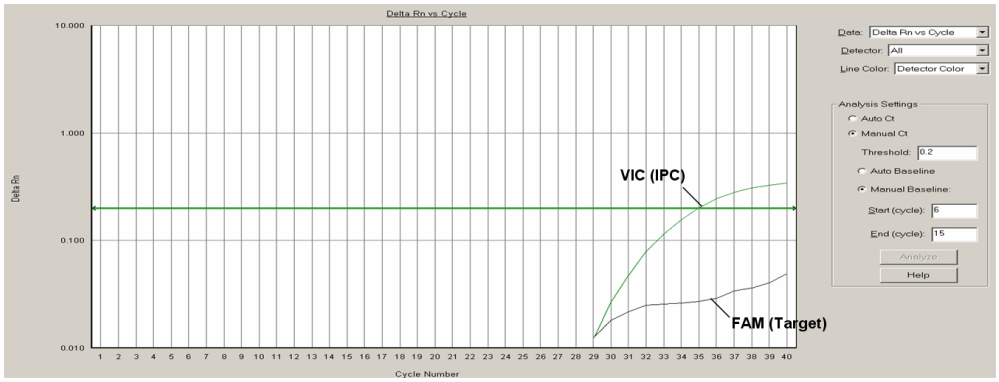


Figure 7 Negative result: FAM absent, VIC present. Amplification plots of target-specific FAM and IPC-specific VIC signals were generated on the 7500 Fast System.

Troubleshooting

Observation	Possible cause	Action
No IPC (VIC) or target-specific (FAM) dye signal is detected in unknown wells	RT (reverse transcription) or PCR inhibited	Repeat the sample preparation, then repeat the assay. If RT or PCR remains inhibited, dilute the sample (for example, 1:5 or 1:10) to dilute inhibitors, or use alternate RNA purification procedure.
	TaqMan® Influenza Assay Beads and reagents not stored properly	Repeat the assay using properly stored assay components. Protect the assay beads from lights.
IPC signal C_T is greater than 36 in unknown or positive controls wells	RT or PCR inhibited	Repeat the sample preparation, then repeat the assay. If RT or PCR remains inhibited, dilute the sample (for example, 1:5 or 1:10) to dilute inhibitors, or use alternate RNA purification procedure.
IPC signal C_T is inconsistent between unknown wells	Your instrument may have intrinsic well-to-well variability	Repeat assay at different position in the heating block.
No IPC signal is detected, but target-specific signal is detected	High copy number of target RNA resulting in preferential amplification of the target-specific RNA	Dilute the sample (for example, 1:5 or 1:10), then repeat assay reactions.
Target-specific signal is detected in negative-control wells	Carryover contamination	Repeat the assay reactions using fresh aliquots of all reagents and clean pipetting equipment. If the negative control still shows contamination, repeat assay reactions using a new kit. If the negative control still shows contamination, contact Applied Biosystems Technical Support.
Target-specific signal and no IPC signal is detected in negative-control wells	Carryover contamination and one of the following: • High copy number of contaminating target RNA resulting in preferential amplification of the target-specific RNA • Problem with IPC amplification	Examine unknowns to determine if IPC signal is present. If IPC signal is present in unknown wells, you can rule out a problem with IPC amplification. Repeat the assay reactions using fresh aliquots of all reagents and clean pipetting equipment.
No IPC or target-specific signal is detected in positive-control wells	TaqMan® Influenza assay beads and reagents not stored properly	Repeat the assay using properly stored assay components. Protect the assay beads from light.
No target-specific signal is detected in positive-control wells	Pipetting error (no positive control added)	Repeat the assay. Make sure to pipette the positive control into all positive control wells.

Observation	Possible cause	Action
Replicate results for sample are inconsistent	Your instrument may have intrinsic well-to-well variability.	If more than two replicates yield the same result (for example, you ran three replicates and two replicates are negative, but one replicate is positive), the result associated with the larger number of replicates is probably accurate. However, your laboratory protocol may dictate that you repeat the assay using fresh samples and reagents. If you ran only two replicates and results are not consistent, repeat the assay using fresh samples and reagents. If instrument variability is suspected, try repeating the assay at a different position in the heating block. In addition, check the lamp status on the instrument.
No target-specific signal detected: background noise crosses the threshold	High background noise	Manually set the threshold slightly above any negative-control signal. However, setting the threshold too high above background noise may result in decreased detection sensitivity of positive samples. If the new threshold does not cross the exponential phase in the positive wells, decrease the threshold. Reanalyze. Note: setting the threshold above the negative control signal changes the C_T of the negative controls to “Undetermined.”
IPC signal C_T is less than 28 and/or ROX fluorescence signal in multicomponent plot clearly decreases over course of reaction (see figure below)	ROX degradation	Repeat the assay reactions.

A B

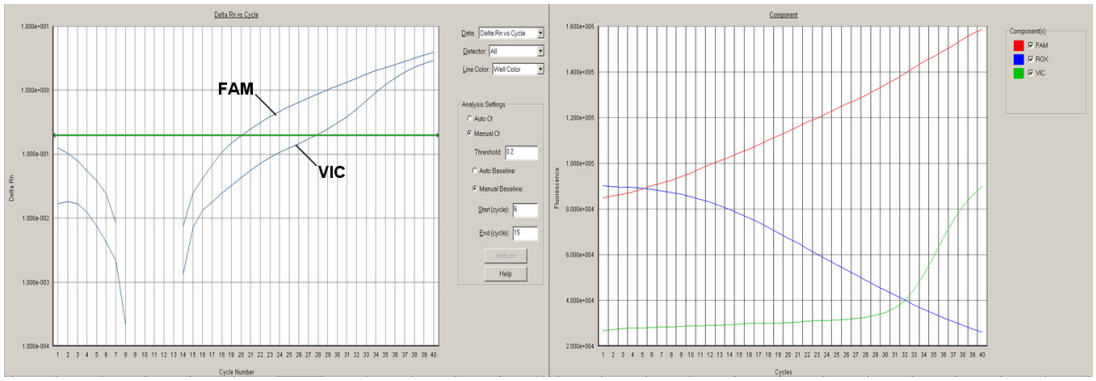


Figure 8 A) Amplification plot of sample where FAM™ and VIC® signals were shifted in response to unusual ROX™ degradation. B) Multicomponent plot showing linear loss of ROX signal (blue curve) that is diagnostic of ROX degradation.

Good PCR Practices

Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

IMPORTANT! To avoid false positives due to cross-contamination:

- Prepare and close all negative-control and unknown sample tubes before pipetting the positive control.
- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.

Appendix B

Safety

This appendix covers:

- Chemical safety 28
- Chemical waste safety 29
- Biological hazard safety 31
- Chemical alerts 31

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 29.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you select

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets (MSDSs) and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

  IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at: **www.cdc.gov**

Chemical alerts

General alerts for all chemicals

Avoid contact with (skin, eyes, and/or clothing). Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! The VLA TaqMan® Influenza A/H5/H7 Detection Kits cause eye, skin, and respiratory tract irritation. Avoid breathing dust. Use with adequate ventilation. Avoid contact with eyes and skin. Read MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Related documentation

For additional documentation, see "How to obtain support" on page viii.

Real-time PCR system	Document	PN
All real-time PCR systems	*VLA TaqMan Influenza A/H5/H7 Detection Kits Quick Reference Card*	4400541
7900 Fast System	*Applied Biosystems 7900 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide*	4364014
7300, 7500, and 7500 Fast Systems	*Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide*	4347825
StepOne and StepOnePlus Systems	*Applied Biosystems StepOne and StepOnePlus Real-Time PCR Systems Presence/Absence Experiments Getting Started Guide*	4376787

Portable document format (PDF) versions of this guide and the documents listed above are available at **www.appliedbiosystems.com**

Note: To open the documentation available from the Applied Biosystems web site, use the Adobe® Acrobat® Reader® software available at **www.adobe.com**

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